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### (54) Oral care compositions

(57) The invention relates to oral care compositions with colloidal anti-plaque agents which have been coated with particular polymers containing carbohydrate (saccharide) or peptide structures which are specifically recognised by bacterial adhesins, more particularly carbohydrate containing polymers recognized by bacterial lectins, or with antibodies or antibody fragments which recognize plaque bacterial antigens. Such coated colloidal anti-plaque agents, e.g. coated colloidal zinc oxide, can be targeted to specific sites in the human mouth where the anti-plaque agent is then released at acidic pH.

EP 0 737 470 A1

**Description****Field of the Invention**

5 The present invention relates to the delivery of colloidal anti-plaque agents to dental plaque by the use of

- (i) antibodies which specifically recognise plaque bacterial antigens (e.g. *Streptococcus sanguis*, *Streptococcus mutans* etc) and/or
- (ii) polymers containing carbohydrate (saccharide) or peptide structures which are specifically recognised by bacterial adhesions, more particularly carbohydrate containing polymers recognized by carbohydrate binding proteins ie lectins. The invention also relates to oral care compositions comprising such delivery systems.

**Background of the Invention**

15 The adherence of bacteria to the enamel pellicle and the polysaccharides that they metabolise generally leads to the development of plaque (a microbial community embedded in polymers of salivary and bacterial origin) on the tooth surface. In the absence of good oral hygiene plaque can accumulate beyond levels compatible with oral health which frequently results in a disease state for example caries and gingivitis. Research to date suggests that microbial colonisation is enhanced by stereospecific interactions between cell surface proteins referred to as 'adhesions' and cognate

20 binding sites present for example on other bacteria or on host tissue (Tsivion Y and Sharon N(1981) *Biochim. Biophys. Acta* 642,336; Kawai Y and Yano I(1983) *Eur. J. Biochem.* 136,351; Mirelman D and Ofek I(1986) *In: Microbial Lectins and Agglutinins*, Mirelman D. Ed., John Wiley and Sons. Indeed, many oral species have been shown to possess adhesions which are typically lectins ie carbohydrate binding proteins (Levine *et al.*, 1978 *Infect. Immun.* 19,107; Weerkamp and McBride, 1980 *Infect. Immun.* 30, 150). Oral bacterial lectins which mediate attachment to carbohydrate structures

25 present on pellicle, plaque matrix and bacterial surfaces undoubtedly enhance the development of plaque.

The present invention relates to the targeted delivery of colloidal anti-plaque agents to the tooth surface. The term "anti-plaque agents" as used herein covers antimicrobial agents which kill certain oral bacteria such as *S. sanguis* or *S. mutans*, as well as agents that prevent or reduce plaque formation in another way, e.g. by influencing plaque pH or by forming antimicrobial agents *in situ*. Typical examples of antimicrobial agents are antimicrobial metals and metal oxides, such as silver, copper, zinc, iron, tin, mercury, lanthanum, yttrium, indium, gold. A preferred antimicrobial agent is zinc oxide, and the invention will be further discussed and illustrated on the basis of zinc oxide, it being understood, however, that the invention is not limited thereto.

Examples of other anti-plaque agents are plaque-pH buffering agents such as chalk, and agents that form antimicrobial agents *in situ*, e.g. oxidoreductases, which are adsorbed onto a colloidal carrier material such as colloidal silica.

35 Colloidal zinc oxide is potentially a dual functional agent where the release of zinc ions at acidic pH results in (i)inhibition of microbial glycolysis and (ii) a buffering effect. An additional advantage of using colloidal zinc oxide is its small particulate size (ca. 10nm), which enhances its diffusion through plaque and as a consequence its efficacy. Particulate zinc oxide can be targeted to a specific site by e.g. (i) polymers, particularly glycoproteins and other saccharide or oligosaccharide containing adducts, and (ii) antibodies.

40 Several studies have illustrated the anti-plaque activity of zinc and zinc oxide and the ability of the zinc ion to inhibit microbial glycolysis (e.g. Gilbert R J and Ingram G S(1988) *J. Pharm. Pharmacol.* 40,399; Scheie A A;Assar S and Rolla G (1988) *APMIS* 96,761; JP 4038904; WO 8700051 and DE 3681289). However, colloidal zinc oxide has not been proposed for this purpose nor has it been suggested to deliver it to a site by a specific targeting system. Another proposed dental application includes the use of zinc oxide as a hardenable and/or cushioning material for treatment of caries (e.g. No. EP 329 098; SU 1648473). Also, zinc oxide has been proposed as a carrier of monoclonal antibodies in diagnostic methods (e.g. JP 5010952).

50 One of the major technical problems associated with the development of effective active systems for oral care benefits is obtaining substantive delivery of the active to the desired site. Most anti-plaque agents which are currently used bind to oral surfaces via (i)electrostatic and/or (ii) hydrophobic interactions. However, this binding is generally reversible and non-specific in nature, ie agents will bind to all oral tissues. The substantivity of the actives of the present invention will be enhanced by specific targeting to sites which can serve as anchor points within the mouth, followed by controlled release of zinc ions at an acidic pH.

55 Complexes can be formed between colloidal zinc oxide and the targeting system: eg antibodies, polymers. Compounds can be targeted to the tooth surface to control the development of bacteria associated with caries. Targeting and thus substantivity leads to a reduced amount of 'active' required. The targeting groups can be generated by synthetic chemistry (eg polymers, antibodies), purification or extraction from natural sources (eg. milk proteins) or through the tools of molecular biology (eg. antibodies and fragments).

## Detailed description of the invention

The purpose of the present invention is to use specific targeting systems (eg. polymers, antibodies) for the substantive delivery of colloidal anti plaque agents, more particularly zinc oxide, to plaque bacteria.

5 In the case of a polymer targeting system, polymers which contain structures specifically recognised by bacterial adhesions are used to deliver zinc oxide. This is achieved by coating zinc oxide particles with the polymer. Such polymer targeting systems include natural or synthetic polymers containing saccharide or peptide structures specifically recognised by bacterial adhesions. Synthetic polymers include polyanionic, polycationic, or amphoteric adducts with: i) specific saccharides or oligosaccharides residues such as lactobionamide, maltobionamide, and other sugar structures; or ii) peptide structures. Natural polymers include dextrans, starches, bovine glycoproteins, plant glycoproteins and polysaccharides, and other natural macromolecules with sugar groups recognised by plaque bacteria. Furthermore, mixtures of proteins can be used to deliver colloidal metal oxide complexes. Suitable polymers include eg. dextrans (mentioned above), fetuin, food grade glycoprotein from bovine milk such as  $\kappa$ -casein, asialo- $\kappa$ -casein, sweet whey, and asialofetuin.

10 15 In the case of an antibody targeting system an antibody or antibody fragment will bind to a target site (for example antibodies raised against *S. sanguis*, *S. mutans* will bind to their respective antigen ie *S. sanguis*, *S. mutans*). Antibody targeting systems include whole antibodies or monovalent or polyvalent fragments of antibodies which are derived synthetically or using molecular biology and natural production systems such as fermentation. Complexes may be formed through chemical complexation between anti-bacterial antibodies (fragments) and colloidal zinc oxide. Alternatively, a 20 fusion can be made which consists of the antibody fragment binding region and colloidal zinc oxide. Antibody fragment fusions with peptide structures which adsorb efficiently to colloidal metal oxide complexes can also be used. Furthermore, self assembly systems can be utilised to target colloidal metal oxides, whereby a primary plaque-specific antibody or fragment is utilised to target plaque structures, and secondary antibodies (or fragments) specific for the first antibody (or fragment) are used to coat the metal oxide complex. In this way the dual antibody systems assemble in situ 25 to target the complex to specific structures in dental plaque.

Another object of this invention is to use polymers which contain carbohydrate residues, specifically recognised by bacterial lectins, to target colloidal zinc oxide to a specific site and release zinc ions at an acidic pH. Another object of this invention is to use antibodies which recognise plaque bacteria to target colloidal zinc oxide to a specific site and release zinc ions at acidic pH.

30 Targeted colloidal zinc oxide described herein can be formulated into dentrifrices, mouthrinses, gels, dental flosses and other oral care products.

The oral care products can be in the form of toothpastes, gels, mouthwashes, powders, gargles, lozenges, chewing gum and the like.

35 The oral composition may furthermore comprise conventional ingredients, such as pharmaceutically acceptable carriers like starch, sucrose, polyols, surfactants, water or water/alcohol systems etc. When formulated into a dentifrice, such formulation may contain all the usual dentifrice ingredients. Thus, they may comprise particulate abrasive materials such as silicas, aluminas, calcium carbonates, dicalciumphosphates, hydroxyapatites, calcium pyrophosphates, trimetaphosphates, insoluble hexametaphosphates and so on, usually in amounts between 5 and 60% by weight.

40 Furthermore, the dentifrice formulations may comprise humectants such as glycerol, sorbitol, propylene glycol, latitol and so on.

Surface-active agents may also be included such as anionic, nonionic, amphoteric and zwitterionic synthetic detergents. Examples thereof are sodium lauryl sulphate, sodium dodecylbenzenesulphonate, sodium mono- and dioctylphosphate, sodium lauroylsarcosinate, cocamidopropylbetain.

45 Binders and thickeners such as sodium carboxymethylcellulose, xanthan gum, gum arabic etc., may also be included, as well as synthetic polymers such as polyacrylates and carboxyvinyl polymers such as Carbopol<sup>®</sup>.

Flavours such as peppermint and spearmint oils may also be included, as well as preservatives, opacifying agents, colouring agents, pH-adjusting agents, sweetening agents and so on.

50 Additional anti-bacterial agents may also be included such as Triclosan, chlorhexidine, copper, zinc- and stannous salts, such as copper sulphate, zinc citrate and stannous pyrophosphate, sanguinarine extract, metronidazole. Further examples of additional anti-bacterial agents are quaternary ammonium compounds such as cetylpyridinium chloride; bis-guanides such as chlorhexidine digluconate, hexetidine, octenidine, alexidine, halogenated bisphenolic compounds such as 2,2'methylenebis-(4-chloro-6-bromophenol).

55 Polymeric compounds which can enhance the delivery of active ingredients such as the anti-bacterial agents can also be included. Examples of such polymers are copolymers of polyvinylmethylether with maleic anhydride and other similar delivery enhancing polymers, e.g. those described in DE-A-3,942,643 (Colgate).

Furthermore anti-inflammatory agents such as ibuprofen, flurbiprofen, aspirin, indomethacin etc., may also be included.

Anti-caries agents such as sodium- and stannous fluoride, aminefluorides, sodium monofluorophosphate, calcium lactate and/or calcium glycerophosphates, strontium salts and strontium polyacrylates, casein and casein digests and phosphoproteins may also be included.

5 Other optional ingredients include vitamins such as Vitamin C, plant extract, potassium salts such as potassium citrate, potassium chloride and potassium nitrate.

Other optional ingredients include enzymes such as dextranase and/or mutanase, amyloglucosidase, glucose-oxidase with lactoperoxidase, neuraminidases, and hydrogen peroxide generating compounds such as potassium peroxylipophosphate.

10 Furthermore, the oral compositions may comprise anti-calculus agents such as alkali metal pyrophosphates, hypophosphite-containing polymers, organic phosphonates, phosphocitrates, etc.

Other optional ingredients that may be included are e.g. bacteriocins, bacteriophages, tissue respiratory factors, antibodies, bleaching agents such as peroxy compounds, effervescent systems such as sodium bicarbonate/citric acid systems, colour change systems, and so on.

15 The present invention will be further illustrated by way of the examples, of which Example 1 only confirms binding studies of various micro-organisms to various polymers reported in the literature.

### Example 1

#### Carbohydrate (polymer) Target System

20 1) Selection of a polymer to coat zinc oxide.

Several agglutination assays (1.1.1.2) were used to study well documented lectin-receptor interactions of *Actinomyces naeslundii*, *Streptococcus sobrinus* and *Streptococcus sanguis* (as illustrated in table 1). Essentially, these assays monitor optical density (OD) over time where a reduction in OD correlates with an increase in bacterial agglutination, indicative of a lectin-receptor interaction.

30 **Table 1: Carbohydrate structures present in certain polymers which are recognised by lectins of the three oral species.**

35

**Table 1**

	Bacterium	Carbohydrate Structure	Polymer
40	Lectin For		
	----->		
45	<i>S. sobrinus</i>	$\alpha$ 1,6 glucose residues	Dextrans >70KDa
50	<i>S. sanguis</i>	N-acetyl neuraminic acid (sialic acid)	Fetuin
	<i>A. naeslundii</i>	$\beta$ -galactose	Asialofetuin

55

#### 1.1 Latex Bead Agglutination

This assay was used to study surface binding of *A. naeslundii* and *S. sanguis* to polymer coated beads.

## (i) Preparation of micro-organisms

5 Cultures of *A. naeslundii* PK 29, *S. sanguis* G9B and *S. sobrinus* 6715 were grown up overnight at 37°C in brain heart infusion supplemented media (brain heart infusion 47g/l; yeast extract 5g/l; cysteine HCL 0.1g/l; haemin 500ml/l; vitamin K 5mg/l; sterile horse blood 50ml/l). Bacterial cultures were then centrifuged (2000g; 10 minutes), washed twice in phosphate buffered saline (PBS) and resuspended in PBS to an appropriate absorbance at 600 nm.

## (ii) Preparation of polymer coated latex beads

10 200 µl of latex beads (diameter 6.4 µ, Sigma, Catalogue No.SD-6A) were dispensed into a polypropylene tube (Greiner) containing a 20mM Trixma based (Sigma) buffer, pH 8.2 (0.73% glycine; 1% sodium chloride; 0.1mM calcium chloride and 0.02% sodium azide 'TGS') and 5mg of an appropriate coating glycoprotein (ie asialofetuin : *A. naeslundii*; fetuin : *S. sanguis*). The sample was incubated at 37°C for 1 hour on a rotary mixer (60 rpm, Heidolph) and then washed and centrifuged three times in TGS buffer + 0.1% bovine serum albumin (BSA). Finally, the deposit was resuspended in 10 ml of 0.1% BSA/TGS buffer.

## (iii) Assay procedure

20 Disposable polystyrene cuvettes with sealable plastic caps (Sigma Aldrich Techware) containing a total sample volume of 2.0 ml were used. 1.0 ml of latex treated (asialofetuin or fetuin) beads were placed in the cuvette followed by an appropriate amount of TGS. Finally, 0.1 ml of the bacterial suspension \* (*A. naeslundii* or *S. sanguis*) was added to initiate agglutination. The cuvettes were sealed and agitated prior to an initial absorbance reading.

Subsequent readings were taken at two minute intervals for a twenty minute period (OD 600nm). Cuvettes were rotated at 25rpm between readings.

25 NB. Control samples contained latex beads which were coated with the non-carbohydrate protein BSA which would not be recognised by the bacterial lectin. Specificity of the reaction was determined by incorporating lactose, a β-galactoside, which competes for the bacterial lectin site on *A. naeslundii* and inhibits agglutination.

## Results

30 Incubation of asialofetuin treated latex beads with *A. naeslundii* resulted in a large drop in absorbance with a concomitant increase in agglutination (figure 1). Here,bacterial agglutination was attributed to interaction of the bacterial lectin with multiple β-galactose structures on the latex beads. Specificity of the bacterial binding is indicated by the lactose results where the addition of lactose,which competes for the bacterial lectin site, appears to have bound to the lectin site on *A. naeslundii* and inhibited bacterial agglutination. Glucose which does not compete for the lectin site had little or no effect on bacterial agglutination.

35 When *S. sanguis* was added to fetuin coated latex beads there was little/no bacterial agglutination. This was an unexpected result as *S. sanguis* has a well documented lectin for sialic acid residues, in this instance purported to be present in fetuin. The lack of agglutination is most likely attributed to the manufacturing process of fetuin where a large proportion of the terminal sugar ie sialic acid may have been cleaved off and lost in the process.

## 1.2 Polymer-Induced Agglutination

40 This assay was used to study glucan binding of *S. sobrinus* where bacterial agglutination would be expected to occur if recognition occurred between α-1,6 linked glucose residues in dextrans of greater than 70KDa and the lectin of *S. sobrinus*.

## Assay procedure

45 Disposable cuvettes, as before, were used for this assay. 0.2 ml of 5% BSA in PBS was dispensed into a cuvette followed by the addition of 1.6 ml of bacterial suspension\*. The agglutinin (dextrans > 70 KDa, in this instance 79 KDa and 2000 KDa) was then added to the suspension which was brought to a total volume of 2.0 ml by the addition of PBS. The cuvettes were sealed and agitated prior to an initial absorbance reading. Subsequent readings were taken at two minute intervals for a twenty minute period (OD 600nm).Cuvettes were rotated at 25 rpm between readings. Controls included (a) bacteria without dextran and (b) bacteria with dextrans of < 70 KDa where bacterial agglutination would not be expected.

\* Final OD (600nm) of bacterial suspensions was 1.0.

\* Final OD (600nm) of bacterial suspensions was 1.0.

## Results

5 The addition of *S. sobrinus* to dextrans of 79 KDa and 2000 KDa caused a drop in absorbance which corresponded to an increase in bacterial agglutination (figure 2/4). This response suggested lectin recognition of glucose residues in dextrans of greater than 70 KDa. No bacterial agglutination was observed when *S. sobrinus* was added to dextrans of less than 70 KDa. Dextrans less than ca. 70 Kda are presumably not large enough to crosslink multiple bacteria and therefore are not expected to induce agglutination.

## 1.3 Polymer Selection

10 The polymer selected to coat ZnO, on the basis of the above studies, was one that resulted in rapid, specific agglutination. The polymer found to meet such criteria was the  $\beta$ -galactose containing glycoprotein asialofetuin as depicted in figure 1/4.

## 15 Example 2

Bacterial targeting of zinc oxide with  $\beta$ -galactose containing glycoprotein (asialofetuin)

## 2.1 Agglutination Assay

## 20 Method

## (i) Preparation of colloidal zinc oxide (primary particle size of ca.10nm)

25 A coordination precursor complex to zinc oxide was synthesised by refluxing a 0.1M solution of  $Zn(OAc)_2 \cdot 2H_2O$  in ethanol for 3 hours. The solution was cooled to 0°C prior to the addition of an ethanolic solution of NaOH which made the final concentration of base 0.1M. The solution was stirred for 1 hour and then evaporated to dryness. The resulting white solid was washed with distilled water, freeze dried and calcined at 160°C to remove any loosely bound organics, particularly acetic acid.

## 30 (ii) Preparation of asialofetuin coated zinc oxide

Asialofetuin (0.02%) was added to a 0.03% solution of zinc oxide in distilled water and shaken on a roller mixer (Denley, Spiramix 5) overnight (RT).

## 35 (iii) Assay procedure

40 Polystyrene cuvettes containing a total sample volume of 2.0 ml were used. 1.0 ml of asialofetuin coated zinc oxide was placed in the cuvette followed by an appropriate amount of TGS buffer. The bacterial suspension\* (0.1 ml of *A. naeslundii* or *S. sanguis*) was then added to initiate agglutination. The cuvettes were sealed and agitated prior to an initial absorbance reading. Subsequent readings were taken at two minute intervals for a twenty minute period (OD 600nm). Cuvettes were rotated at 25 rpm between readings.

45 NB. Control samples contained zinc oxide which was coated with the non-carbohydrate BSA which would not be recognised by the above bacterial lectins.

Specificity was determined by the addition of lactose which competes for the bacterial lectin site and as a consequence inhibits agglutination.

## Results

50 Results indicated a specific interaction (ie lactose inhibitable) between the bacterial lectin of *A. naeslundii* and the  $\beta$ -galactose structures on the asialofetuin coated zinc oxide as illustrated in figure 3/4.

55

\*Final OD (600nm) of bacterial suspensions was 1.0.

## 2.2 Transmission Electron Microscopy(TEM)

### Method

5 TEM was used to visualise the association of zinc with *A. naeslundii* within bacterial aggregates. Samples of test (asialofetuin coated zinc oxide + *A. naeslundii*) and controls (BSA coated zinc oxide + *A. naeslundii*; asialofetuin + lactose + *A. naeslundii*) were dispensed onto transmission electron microscopy grids prior to viewing on a JEOL 200CX TEM at 80keV.

### 10 Results

There appeared to be a clear difference with respect to zinc deposition between the test and control samples where sparse zinc was observed in control samples compared to abundant zinc (glomerates up to 0.7 $\mu$ ) on and around bacterial aggregates in test samples. When samples were titrated (asialofetuin coated zinc oxide 1:4; BSA coated zinc oxide 15 1:4) zinc was not observed in control samples

Results illustrated the ability of  $\beta$ -galactose residues, present on asialofetuin, to target colloidal zinc oxide to a specific site on *A. naeslundii*.

Figure 3/6 shows  $\beta$ -galactose specific agglutination of *A. naeslundii* by asialofetuin coated zinc oxide.

### 20 Example 3

#### Delivery of zinc ions from asialofetuin coated ZnO

### Method

25 *S. mutans* (an organism which produces acid from the metabolism of carbohydrates and has been associated with caries) was grown up overnight in BHI (15% CO<sub>2</sub>, 37°C) and an inoculum of this culture made into fresh BHI (diluted 1/3) containing 4% sucrose. Cultures were incubated (15% CO<sub>2</sub>, 37°C) and samples taken at time 0 and then at hourly intervals (up to 9 hours). An equal volume of the bacterial aggregate (ie asialofetuin coated zinc oxide and *A. naeslundii*) 30 containing 2mM of zinc oxide was added to the *S. mutans* suspension prior to incubation for up to 1 hour at 37°C. After this period samples were taken for pH determination and viable counts (CFU/ml).

### Results

35 The addition of zinc oxide aggregates to cultures of *S. mutans* had very little effect on either pH or viable count until the culture pH dropped to pH 4.29. At this pH (7 hours growth) a significant effect on pH and viable count of *S. mutans* was observed where the addition of the zinc oxide aggregate resulted in an increase in culture pH from 4.29 to 5.45 and a reduction in viable count of *S. mutans* (tables 2 and 3).

### 40

Table 2

Effect of zinc oxide aggregate on culture pH of <i>S. mutans</i>	
	Culture pH
Test (+ 2mM ZnO)	5.45
Control (- ZnO)	4.29
(No significant difference with respect to pH was observed if the ZnO aggregate was added to the culture of <i>S. mutans</i> for 10 minutes or for up to 1 hour).	

### 55

Table 3

Effect of the zinc oxide aggregate on viable count of <i>S. mutans</i> .		
	Viable Count	
	CFU/ml	Log
Test (+ 2mM ZnO)	$2.4 \times 10^7$	7.38
Control (- ZnO)	$4.2 \times 10^8$	8.62

15 The reduction in viable count of *S. mutans* (as shown in table 3) is probably due to inhibition of glucose metabolism as a consequence of zinc ion release at acidic pH.

#### Example 4

##### 20 Targeting of zinc oxide with antibacterial antibody

###### 4.1 Methods

###### (i) Preparation of antibody coated zinc oxide

25 Colloidal zinc oxide (0.03%) was added to a 0.25 mg/ml solution of antibacterial antibody (i.e. anti *Streptococcus sanguis* IgG) in Tris/HCl buffer, pH 9.1. This suspension was mildly sonicated prior to end mixing on a rotary shaker (60rpm, Heidolph) for 5 hours. The suspension was then washed and centrifuged (10,000 rpm: 20 seconds, Eppendorf centrifuge) three times in Tris/HCl buffer to remove any excess antibody. Finally the pellet was resuspended in 1ml of 30 buffer and stored at 4°C.

###### (ii) Specificity of antibody coated zinc oxide

35 An immunofluorescent method which employs confocal laser scanning microscopy (CLSM) was used to determine specificity ie the ability of the antibody coated zinc oxide to recognise its antigen (figure 4/4). This method which quantifies the antibody-antigen reaction by pixel intensity allows *in-situ* viewing of the sample. Briefly, the antigen (*S. sanguis*: prepared as in 1.1) was added to saliva coated hydroxyapatite discs prior to the addition of a blocking agent (BSA) to reduce non-specific reactions. The antibody coated zinc oxide was then added before the addition of a fluorescently labelled antibody (Vector). Finally, the discs were viewed under the CLSM where digital images were produced and quantification on the basis of pixel intensity (20 images/test) determined. Settings used on the CLSM were 40 : pinhole 150; voltage 900; offset -10; magnification x 100.

###### 4.2 Results

45 The antibacterial (*S. sanguis*) antibody coated zinc oxide appeared to be specific where the pixel intensity result indicated strong antigen recognition (table 4).

Table 4

	Antibody Binding Pixel Intensity*
<i>S. sanguis</i> antibody coated ZnO	161.12 (+/- 45.82)
Negative control (no anti-bacterial antibody)	14.64 (+/- 4.97)

50 55 \* = measure of fluorescence

## Example 5

## Delivery of zinc ions from antibody-coated zinc oxide

## 5 5.1 Method

10 *S. mutans* was grown up overnight in BHI (15%CO<sub>2</sub>, 37°C) and an inoculum of this culture made into fresh BHI (diluted 1/3) containing 4% sucrose. Cultures were incubated (15%CO<sub>2</sub>, 37°C) and samples taken at time 0 and then at hourly intervals (up to 9 hours). An equal volume of antigen (*S. sanguis*: see 1.1 (i) for preparation) was added to the bacterial suspension followed by the antibody coated zinc oxide containing 2 mM of zinc oxide. Samples were then incubated for up to 1 hour at 37°C. After this period samples were taken for pH determination and viable counts (CFU/ml).

## 15 5.2 Results

15 Similar to the polymer delivery work (see Example 3) the addition of antibody coated zinc oxide to cultures of *S. mutans* had very little effect until the culture pH dropped to pH 4.32. At this pH (7 hours growth) a significant effect on pH and viable counts was observed where the addition of antibody coated zinc oxide resulted in an increase in culture pH from 4.32 to 5.92 and a reduction in viable count of *S. mutans*.

20

Table 5

Effect of antibody coated ZnO on culture pH of <i>S. mutans</i>	
	Culture pH
Test (+ 2mM ZnO)	5.92
Control (- ZnO)	4.32
(No significant difference with respect to pH was observed if the antibody coated zinc oxide was added to the culture of <i>S. mutans</i> for 10 minutes or for up to 1 hour).	

35

Table 6

Effect of antibody coated zinc oxide on viable count of <i>S. mutans</i>		
	Viable Count	
	CFU/ml	Log
Test (+ 2mM ZnO)	6.5x10 <sup>6</sup>	6.81
Control (- ZnO)	2.9x10 <sup>8</sup>	8.46

50

50 The reduction in viable count of *S. mutans* (as shown in table 6) is probably due to inhibition of glucose metabolism as a consequence of zinc ion release at acidic pH.

## Claims

55

1. An oral composition comprising an anti-plaque agent, characterised in that the anti-plaque agent is a colloidal anti-plaque agent, coated with a polymer which contains carbohydrate or peptide structures which are specifically recognised by bacterial adhesins, or coated with an antibody or antibody fragment which recognizes plaque bacterial antigens.

2. An oral composition according to claim 1, wherein the colloidal anti-plaque agent is an anti-microbial metal or oxide thereof.
3. An oral composition according to claim 2, wherein the colloidal anti-plaque agent is colloidal zinc oxide.
- 5 4. An oral composition according to claim 1-3, wherein the polymer contains carbohydrate structures which are specifically recognized by bacterial lectins.
- 10 5. An oral composition according to claim 4, wherein the polymer is the milk glycoprotein or glycoprotein mixture:  $\kappa$ -casein, asialo- $\kappa$ -casein, sweet whey, and asialofetuin.
6. An oral composition according to claims 1-3, wherein the colloidal anti-plaque agent is coated with an antibody or antibody fragment, raised against oral bacteria.
- 15 7. Use of colloidal anti-plaque agents, coated with a polymer which expresses carbohydrate-structures specifically recognised by bacterial lectins, or coated with an antibody or antibody fragment which recognizes plaque bacterial antigens, to target the colloidal anti-plaque agent to specific sites in the human mouth to release the colloidal anti plaque agent *in-situ*.

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25

30

35

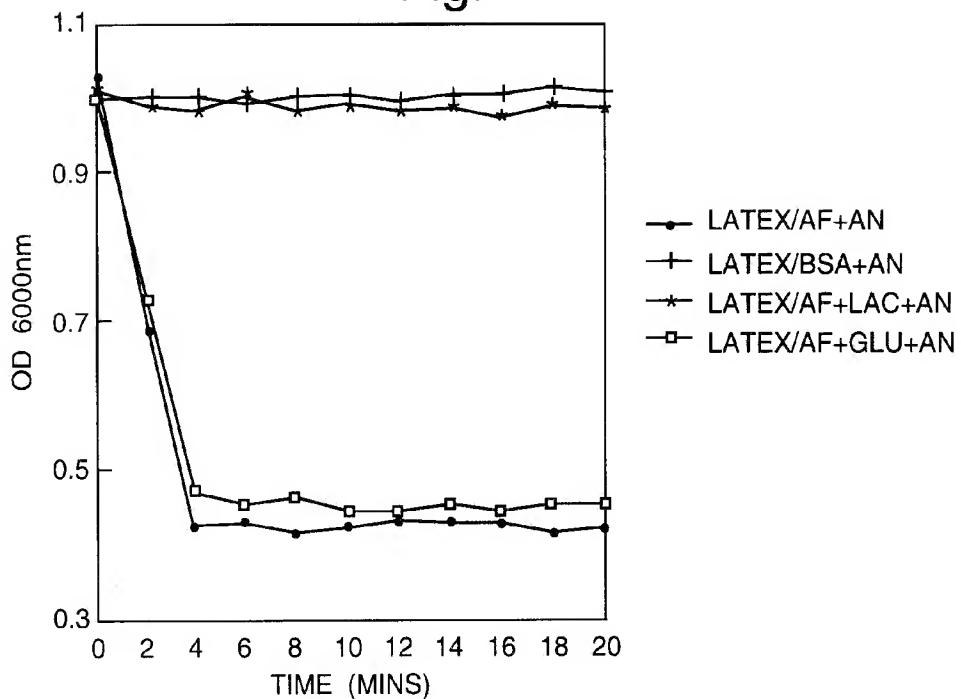
40

45

50

55

Fig.1.



AF=ASIALOFETUIN  
 AN=A.NAESLUNDII  
 LAC=LACTOSE  
 GLU=GLUCOSE

Fig.2.

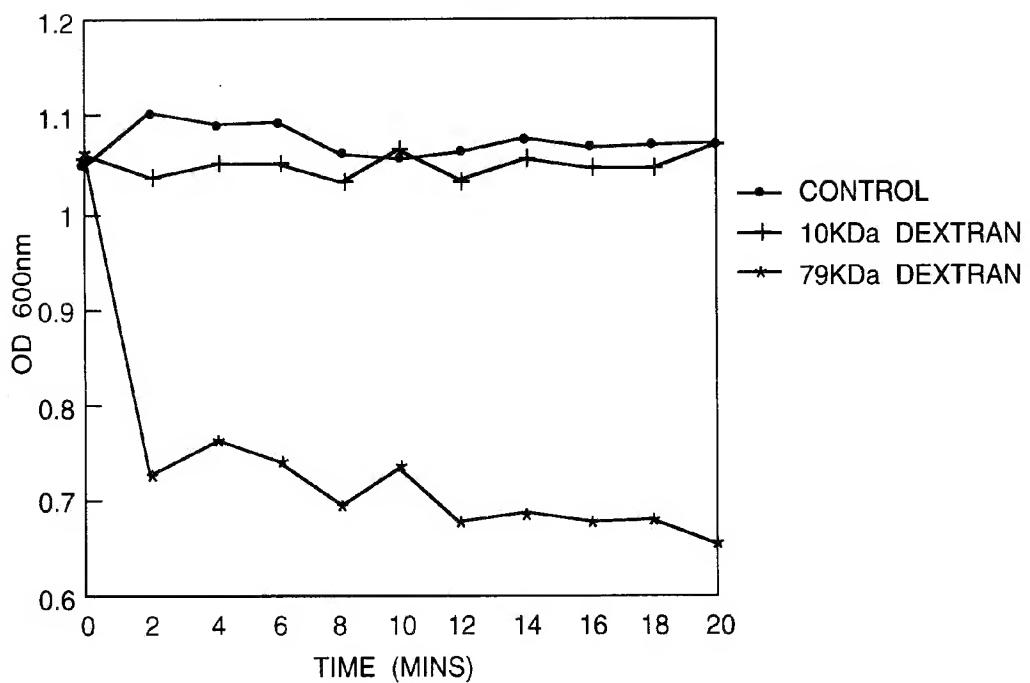


Fig.3.

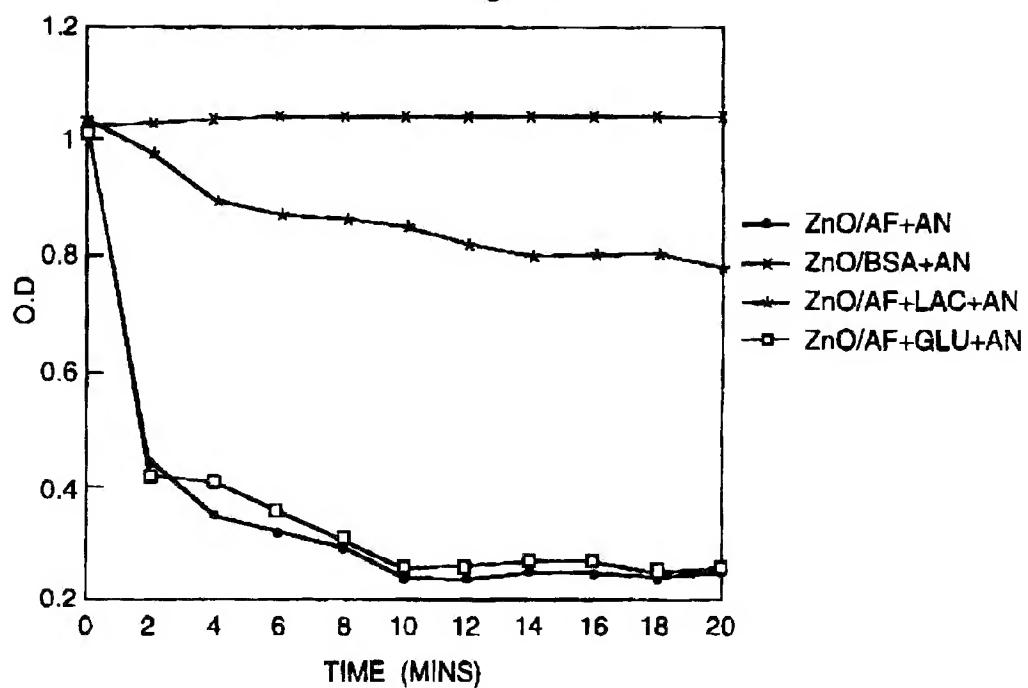
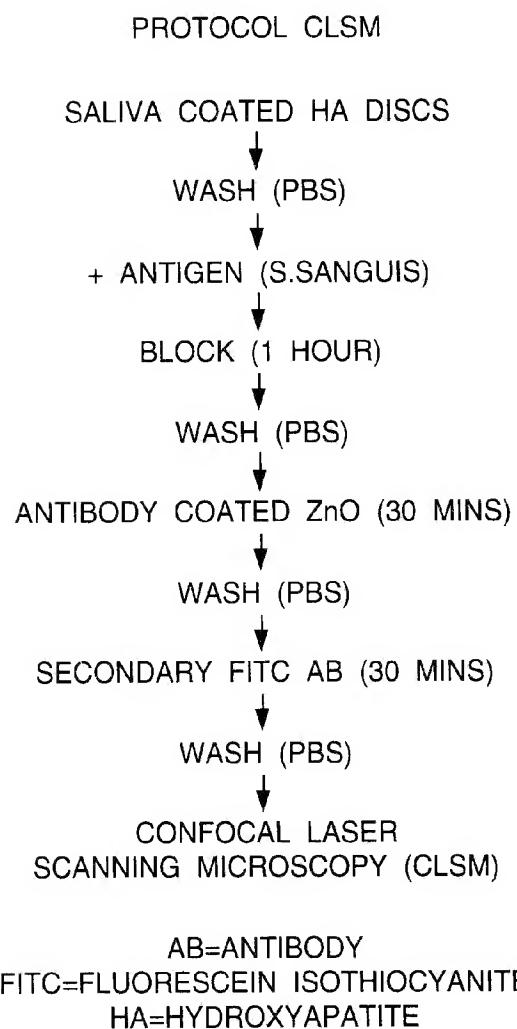


Fig.4.





European Patent  
Office

## EUROPEAN SEARCH REPORT

Application Number  
EP 96 20 0945

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A	EP-A-0 481 701 (UNILEVER) * page 3, line 16 - line 36; claims 1-12 *	1-7	A61K7/16
A	WO-A-94 26245 (CHURCH & DWIGHT) * claims 1-20 *	1-7	
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			TECHNICAL FIELDS SEARCHED (Int.Cl.)
			A61K
<p>The present search report has been drawn up for all claims</p>			
Place of search	Date of completion of the search	Examiner	
THE HAGUE	14 August 1996	Willekens, G	
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ..... & : member of the same patent family, corresponding document	
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document			